REMARKS

Claims 75-78, 80, 102, and 105-108 are pending in this application and under examination. No claims are amended in this response.

Applicants acknowledge with gratitude withdrawal of all previous rejections except for obviousness-type double patenting rejections. Applicants also thank the Examiner for considering the information accompanying the Information Disclosure Statement filed on July 27, 2006.

Applicants' response to the double patenting rejections is as follows:

I. Claims 75-78 and 105-108 were provisionally rejected for obviousness-type double patenting over claims of copending Application No. 10/054,295 (now U.S. Patent 6,921,664). (Office Action paragraph 6.) This rejection is addressed by a terminal disclaimer filed September 29, 2006 (copy enclosed).

II. Claims 75-78 and 105-108 were provisionally rejected for obviousness-type double patenting over claims of Patent No. 6,475,789. (Office Action paragraph 14). This rejection is addressed by a terminal disclaimer filed September 29, 2006 (copy enclosed).

III. Claims 75-78 and 105-108 were rejected for obviousness-type double patenting over claims of Patent No. 6,261,836. (Office Action paragraph 15.) This rejection is addressed by a terminal disclaimer filed September 29, 2006 (copy enclosed).

IV. Claims 75-78 and 105-108 were provisionally rejected for obviousness-type double patenting over claims of copending Application No. 10/044,692. (Office Action paragraph 10). A terminal disclaimer with respect to Application No. 10/044,692 is enclosed, overcoming this rejection.

V. Additionally, a terminal disclaimer were filed on September 29, 2006 with respect to U.S. Pat. Nos. 6,927,285 and 6,337,200 (copy enclosed). A terminal disclaimer

with respect to Application No. 10/044,539 (which has not been cited by the Office) is enclosed herewith.

VI. Claims 75-78 and 105-108 were provisionally rejected for obviousness-type double patenting over claims of copending Application No. 10/877,124. (Office Action paragraph 12.) Copending Application No. 10/877,124 has not yet been examined in the merits, and the present application is expected to issue prior to Application No. 10/877,124. Applicants request that this rejection be withdrawn and applied (if appropriate) during examination of the '124 application.

VII. Claims 75-78 and 105-108 were provisionally rejected for obviousness-type double patenting over claims of copending Application No. 09/721,477. (Office Action paragraph 8.) The pending claims of copending Application No. 09/721,477 stand rejected, and the present application is expected to issue prior to Application No. 09/721,477. Applicants request that this rejection be withdrawn and applied (if appropriate) during examination of the '477 application.

VIII. Claim 75 was rejected for obviousness-type double patenting over claims of Patent No. 6,093,809. (Office Action paragraph 16.) Applicants respectfully traverse this rejection.

U.S. Patent 6,093,809 claims the cDNA sequence of *Euplotes aediculatus* TRT. Accompanying this Response is the article "Reverse transcriptase motifs in the catalytic subunit of telomerase" by Nakamura et al., 1997, Telomerase catalytic subunit homologs from fission yeast and human, *Science* 277:955-9. At page 957, Fig. 2 shows that the hTRT sequence and the *Euplotes* sequence (Ea_p123) have little similarity. Appendix A of this response is a BLAST alignment of the human and *Euplotes* hTRT amino acid sequences. Using this program, only a 586 amino acid region of the hTRT sequence can be aligned with the *Euplotes* sequence, and sequence identity within this region is only about 21%. The longest exact match is six amino acids in length.

The polynucleotide claimed in the '809 patent does not encode at least 100 contiguous amino acids of the hTRT sequence [claims 105-108 of the present application] or encode a protein that has (i) at least 95% identity to hTRT and (ii) motifs required for telomerase catalytic activity [claims 75-78, 80, and 102 of the present application]. Applicants submit the polynucleotides of the instant application are not obvious variants of the polynucleotides of the '809 patent.

IX. Claims 75-78 and 105-108 were rejected for obviousness-type double patenting over claims of Patent No. 6,767,719. (Office Action paragraph 17.) Applicants respectfully traverse this rejection.

U.S. Patent 6,767,719 claims nucleic acids encoding the protein sequence of mouse TRT (mTRT), and certain homologs thereof. Appendix B of this response is a BLAST alignment of the human TRT and mouse TRT sequences at the amino acid level. The human and mouse TRT full length sequences are only about 62% identical. The longest exact match is 22 consecutive amino acids in length.

The polynucleotide claimed in the '719 patent does not encode at least 100 contiguous amino acids of the hTRT sequence [claims 105 -108 of the present application] or encode a protein that has (i) at least 95% identity to hTRT and (ii) motifs required for telomerase catalytic activity [claims 75 -78, 80, and 102 of the present application]. Applicants submit the polynucleotides of the instant application are not obvious variants of the polynucleotides of the '809 patent.

Request for Interview

In the event that the Examiner determines that there are other matters to be addressed before allowance of the application, the undersigned hereby requests an interview by telephone.

Conclusion

All cited patents and applications having been addressed, Applicants respectfully request that the rejections for obviousness-type double patenting be withdrawn, and the application be allowed promptly.

Respectfully submitted,

Randolph Ted Apple
Registration No. 36,429

November 29, 2006

Enclosures:

Copies of terminal disclaimers filed September 29, 2006.
Terminal disclaimer with respect to copending App. Nos. 10/044,692 and 10/044,539.
Nakamura et al., 1997, Telomerase catalytic subunit homologs from fission yeast and human, *Science* 277:955-9.
Appendix A
Appendix B

APPENDIX A: SEQUENCE COMPARISON

Human TRT protein sequence

```
LOCUS       O14746                1132 aa            linear   PRI 15-JUN-2002
DEFINITION  Telomerase reverse transcriptase (Telomerase catalytic subunit)
  ORGANISM  Homo sapiens
  AUTHORS   Nakamura,T.M., Morin,G.B., Chapman,K.B., Weinrich,S.L.,
            Andrews,W.H., Lingner,J., Harley,C.B. and Cech,T.R.
  TITLE     Telomerase catalytic subunit homologs from fission yeast and human
  JOURNAL   Science 277 (5328), 955-959 (1997)

        1 mpraprcrav rsllrshyre vlplatfvrr lgpqgwrlvq rgdpaafral vaqclvcvpw
       61 darpppaaps frqvsclkel varvlqrlce rgaknvlafg falldgargg ppeafttsvr
      121 sylpntvtda lrgsgawgll lrrvgddvlv hllarcalfv lvapscayqv cgpplyqlga
      181 atqarpppha sgprrrlgce rawnhsvrea gvplglpapg arrrggsasr slplpkrprr
      241 gaapepertp vgqgswahpg rtrgpsdrgf cvvsparpae eatslegals gtrhshpsvg
      301 rqhhagppst srpprpwdtp cppvyaetkh flyssgdkeq lrpsfllssl rpsltgarrl
      361 vetiflgsrp wmpgtprrlp rlpqrywqmr plflellgnh aqcpygvllk thcplraavt
      421 paagvcarek pqgsvaapee edtdprrlvq llrqhsspwq vygfvraclr rlvppglwgs
      481 rhnerrflrn tkkfislgkh aklslqeltw kmsvrdcawl rrspgvgcvp aaehrlreei
      541 lakflhwlms vyvvellrsf fyvtettfqk nrlffyrksv wsklqsigir qhlkrvqlre
      601 lseaevrqhr earpalltsr lrfipkpdgl rpivnmdyvv gartfrrekr aerltsrvka
      661 lfsvlnyera rrpgllgasv lglddihraw rtfvlrvraq dpppelyfvk vdvtgaydti
      721 pqdrltevia siikpqntyc vrryavvqka ahghvrkafk shvstltdlq pymrqfvahl
      781 qetsplrdav vieqssslne assglfdvfl rfmchhavri rgksyvqcqg ipqgsilstl
      841 lcslcygdme nklfagirrd gllrlvddf llvtphltha ktflrtlvrg vpeygcvvnl
      901 rktvvnfpve dealggtafv qmpahglfpw cglldtrtl evqsdyssya rtsirasltf
      961 nrgfkagrnm rrklfgvlrl kchslfldlq vnslqtvctn iykilllqay rfhacvlqlp
     1021 fhqqvwknpt fflrvisdta slcysilkak nagmslgakg aagplpseav qwlchqafll
     1081 kltrhrvtyv pllgslrtaq tqlsrklpgt tltaleaaan palpsdfkti ld
```

Euplotes aediculatus TRT protein sequence

```
LOCUS       AAC47515              1031 aa            linear   INV 05-MAY-1997
DEFINITION  telomerase subunit p123 [Euplotes aediculatus].
  ORGANISM  Euplotes aediculatus
REFERENCE   1  (residues 1 to 1031)
  AUTHORS   Lingner,J., Hughes,T.R., Shevchenko,A., Mann,M., Lundblad,V. and
            Cech,T.R.
  TITLE     Reverse transcriptase motifs in the catalytic subunit of telomerase
  JOURNAL   Science 276 (5312), 561-567 (1997)
   PUBMED   9110970

     CDS             1..1031
                     /coded_by="U95964.1:101..3196"
                     /transl_table=10
ORIGIN
        1 mevdvdnqad nhgihsalkt ceeikeaktl yswiqkvirc rnqsqshykd ledikifaqt
       61 nivatprdyn eedfkviark evfstglmie lidkclvell sssdvsdrqk lqcfgfqlkg
      121 nqlakthllt alstqkqyff qdewnqvram ignelfrhly tkylifqrts egtlvqfcgn
      181 nvfdhlkvnd kfdkkqkgga admneprccs tckynvknek dhflnninvp nwnnmksrtr
      241 ifycthfnrn nqffkkhefv snknnisamd raqtiftnif rfnrirkklk dkviekiaym
      301 lekvkdfnfn yyltkscplp enwrerkqki enlinktree kskyyeelfs yttdnkcvtq
      361 fineffynil pkdfltgrnr knfqkkvkky velnkhelih knillekint reiswmqvet
      421 sakhfyyfdh eniyvlwkll rwifedlvvs lircffyvte qqksysktyy yrkniwdvim
      481 kmsiadlkke tlaevqekev eewkkslgfa pgklrlipkk ttfrpimtfn kkivnsdrkt
      541 tkltntkll nshlmlktlk nrmfkdpfgf avfnyddvmk kyeefvckwk qvgqpklffa
      601 tmdiekcyds vnreklstfl kttkllssdf wimtaqilkr knnividskn frkkemkdyf
      661 rqkfqkiale ggqyptlfsv leneqndlna kktliveakq rnyfkkdnll qpvinicqyn
      721 yinfngkfyk qtkgipqglc vssilssfyy atleesslgf lrdesmnpen pnvnllmrlt
      781 ddyllittqe nnavlfiekl invsrengfk fnmkklqtsf plspskfaky gmdsveeqni
      841 vqdycdwigi sidmktlalm pninlriegi lctlnlnmqt kkasmwlkkk lksflmnnit
      901 hyfrktitte dfanktlnkl fisggykymq cakeykdhfk knlamssmid levskiiysv
      961 traffkylvc nikdtifgee hypdfflstl khfieifstk kyifnrvcmi lkakeaklks
     1021 dqcqsliqyd a
```

BLAST COMPARISON — hTRT vs. Euplotes TRT

Source: http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi

Score = 161 bits (407), Expect = 4e-37
Identities = 132/616 (21%), Positives = 257/616 (41%), Gaps = 43/616 (6%)

```
Human:    464  FVRACLRRLVPPGLWGSRHNERRFLRNTKKFISLGKHAKLSLQELTWKMSVRDCAWLRRS 523
               F+      ++P       R N + F +  KK++ L KH  +    L  K++ R+ +W++
EuplA:    361  FINEFFYNILPKDFLTGR-NRKNFQKKVKKYVELNKHELIHKNLLLEKINTREISWMQVE 419

Human:    524  PGVGCVPAAEHRLREEILAKFLHWLMSVYVVELLRSFFYVTETTFQKNRLFFYRKSVWSK 583
                     +H     +L K L W+    VV L+R FFYVTE     ++ ++YRK++W          6 amino acids
EuplA:    420  TSAKHFYYFDHE-NIYVLWKLLRWIFEDLVVSLIRCFFYVTEQQKSYSKTYYYRKNIWDV 478

Human:    584  LQSIGIRQHLKRVQLRELSEAEVRQHREARPALLTSRLRFIPKPDGLRPIVNMDYVVGAR 643
               +  + I   LK+  L E+ E EV + +++       +LR IPK    RPI+  +     +
EuplA:    479  IMKMSIAD-LKKETLAEVQEKEVEEWKKSL-GFAPGKLRLIPKKTTFRPIMTFN----KK 532

Human:    644  TFRREKRAERLTSRVKALFSVLNYERARR---PGLLGASVLGLDDIHRAWRTFVLRVRAQ 700
                  +++  +LT+  K L S L  +  +        G +V   DD+ + +  FV + + Q
EuplA:    533  IVNSDRKTTKLTTNTKLLNSHLMLKTLKNRMFKDPFGFAVFNYDDVMKKYEEFVCKWK-Q 591

Human:    701  DPPPELYFVKVDVTGAYDTIPQDRLTEVI--------------ASIIKPQNTYCVRRYAV 746
                  P+L+F  +D+   YD++ +++L+  +              A I+K +N   +
EuplA:    592  VGQPKLFFATMDIEKCYDSVNREKLSTFLKTTKLLSSDFWIMTAQILKRKNNIVIDSKNF 651

Human:    747  VQKAAHGHVRKAFKSHVSTLTDLQPYMRQFVAHLQETSPLRDAVVIEQSSSLNEASSGLF 806
               +K    + R+ F+  ++      P +   + + Q     +  +++E          L
EuplA:    652  RKKEMKDYFRQKFQK-IALEGGQYPTLFSVLENEQNDLNAKKTLIVEAKQRNYFKKDNLL 710

Human:    807  DVFLRFMCHHAVRIRGKSYVQCQGIPQGSILSTLLCSLCYGDMENKLFAGIRRD------ 860
                  +    ++ +   GK Y Q +GIPQG  +S++L S  Y  +E      +R +
EuplA:    711  QPVINICQYNYINFNGKFYKQTKGIPQGLCVSSILSSFYYATLEESSLGFLRDESMNPEN 770

Human:    861  ---GLLLRLVDDFLLVTPHLTHAKTFLRTLVRGVPEYGCVVNLRKTVVNFPVEDEALGGT 917
                   LL+RL DD+LL+T    +A  F+  L+    E G   N++K   +FP+
EuplA:    771  PNVNLLMRLTDDYLLITTQENNAVLFIEKLINVSRENGFKFNMKKLQTSFPLSPSKFAKY 830

Human:    918  AFVQMPAHGLF----PWCGLLLDTRTLEVQSDYSSYARTSIRASLTFNRGFKAGRNMRRK 973
               +    +      W G+ +D +TL +  + +       I  +L  N   K      +K
EuplA:    831  GMDSVEEQNIVQDYCDWIGISIDMKTLALMPNINLRIE-GILCTLNLNMQTKKASMWLKK 889

Human:    974  LFGVLRLKCHSLFLDLQVNSLQTVCTNIYKILLLQAYRFHACVLQLPFHQQVWKNPTFFL 1033
                    +   + +    + +       + K+ +   Y++  C  +   H +  KN
EuplA:    890  KLKSFLMNNITHYFRKTITTEDFANKTLNKLFISGGYKYMQCAKEYKDHFK--KNLAMSS 947

Human:    1034 RVISDTASLCYSILKA 1049
                +  + + + YS+ +A
EuplA:    948  MIDLEVSKIIYSVTRA 963
```

APPENDIX B: SEQUENCE COMPARISON

Human TRT protein sequence

```
LOCUS       O14746                1132 aa            linear   PRI 15-JUN-2002
DEFINITION  Telomerase reverse transcriptase (Telomerase catalytic subunit)
  ORGANISM  Homo sapiens
  AUTHORS   Nakamura,T.M., Morin,G.B., Chapman,K.B., Weinrich,S.L.,
            Andrews,W.H., Lingner,J., Harley,C.B. and Cech,T.R.
  TITLE     Telomerase catalytic subunit homologs from fission yeast and human
  JOURNAL   Science 277 (5328), 955-959 (1997)

        1 mpraprcrav rsllrshyre vlplatfvrr lgpqgwrlvq rgdpaafral vaqclvcvpw
       61 darpppaaps frqvsclkel varvlqrlce rgaknvlafg falldgargg ppeafttsvr
      121 sylpntvtda lrgsgawgll lrrvgddvlv hllarcalfv lvapscayqv cgpplyqlga
      181 atqarpppha sgprrrlgce rawnhsvrea gvplglpapg arrrggsasr slplpkrprr
      241 gaapepertp vgqgswahpg rtrgpsdrgf cvvsparpae eatslegals gtrhshpsvg
      301 rqhhagppst srpprpwdtp cppvyaetkh flyssgdkeq lrpsfllssl rpsltgarrl
      361 vetiflgsrp wmpgtprrlp rlpqrywqmr plflellgnh aqcpygvllk thcplraavt
      421 paagvcarek pqgsvaapee edtdprrlvq llrqhsspwq vygfvraclr rlvppglwgs
      481 rhnerrflrn tkkfislgkh aklslqeltw kmsvrdcawl rrspgvgcvp aaehrlreei
      541 lakflhwlms vyvvellrsf fyvtettfqk nrlffyrksv wsklqsigir qhlkrvqlre
      601 lseaevrqhr earpalltsr lrfipkpdgl rpivnmdyvv gartfrrekr aerltsrvka
      661 lfsvlnyera rrpgllgasv lglddihraw rtfvlrvraq dpppelyfvk vdvtgaydti
      721 pqdrltevia siikpqntyc vrryavvqka ahghvrkafk shvstltdlq pymrqfvahl
      781 qetsplrdav vieqsssLne assglfdvfl rfmchhavri rgksyvqcqg ipqgsilstl
      841 lcslcygdme nklfagirrd gllLrlvddf llvtphltha ktflrtlvrg vpeygcvvnl
      901 rktvvnfpve dealggtafv qmpahglfpw cglLldtrtl evqsdyssya rtsirasltf
      961 nrgfkagrnm rrklfgvlrl kchslfldlq vnslqtvctn iykilllqay rfhacvlqlp
     1021 fhqqvwknpt fflrvisdta slcysilkak nagmslgakg aagplpseav qwlchqafll
     1081 kltrhrvtyv pllgslrtaq tqlsrklpgt tltaleaaan palpsdfkti ld
```

Mouse TRT protein sequence

```
LOCUS       O70372                1122 aa            linear   ROD 15-JUN-2002
DEFINITION  Telomerase reverse transcriptase (Telomerase catalytic subunit).
  ORGANISM  Mus musculus
  AUTHORS   Greenberg,R.A., Allsopp,R.C., Chin,L., Morin,G.B. and DePinho,R.A.
  TITLE     Expression of mouse telomerase reverse transcriptase during
            development, differentiation and proliferation
  JOURNAL   Oncogene 16 (13), 1723-1730 (1998)

        1 mtraprcpav rsllrsryre vwplatfvrr lgpegrrlvq pgdpkiyrtl vaqclvcmhw
       61 gsqpppadls fhqvsslkel varvvqrlce rnernvlafg fellneargg ppmaftssvr
      121 sylpntviet lrvsgawmll lsrvgddllv yllahcalyl lvppscayqv cgsplyqica
      181 ttdiwpsvsa syrptrpvgr nftnlrflqq iksssrqeap kplalpsrgt krhlsltsts
      241 vpsakkarcy pvprveegph rqvlptpsgk swvpsparsp evptaekdls skgkvsdlsl
      301 sgsvcckhkp sstsllsppr qnafqlrpti etrhflysrg dgqerlnpsf llsnlqpnlt
      361 garrlveiif lgsrprtsgp lcrthrlsrr ywqmrplfqq llvnhaecqy vrllrshcrf
      421 rtanqqvtda lntspphlmd llrlhsspwq vygflraclc kvvsaslwgt rhnerrffkn
      481 lkkfislgky gklslqelmw kmkvedchwl rsspgkdrvp aaehrlreri latflfwlmd
      541 tyvvqllrsf fyitestfqk nrlffyrksv wsklqsigvr qhlervrlre lsqeevrhhq
      601 dtwlampicr lrfipkpngl rpivnmsysm gtralgrrkq aqhftqrlkt lfsmlnyert
      661 khphlmgssv lgmndiyrtw rafvlrvral dqtprmyfvk advtgaydai pqgklvevva
      721 nmirhsesty cirqyavvrr dsqqqvhksf rrqvttlsdl qpymgqflkh lqdsdasalr
      781 nsvvieqsis mnesssslfd fflhflrhsv vkigdrcytq cqgipqgssl stllcslcfg
      841 dmenklfaev qrdglllrfv ddfllvtphl dqaktflstl vhgvpeygcm inlqktvvnf
      901 pvepgtlgga apyqlpahcl fpwcglllDt qtlevfcdys gyaqtsikts ltfqsvfkag
      961 ktmrnkllsv lrlkchglfl dlqvnslqtv ciniykifll qayrfhacvi qlpfdqrvrk
     1021 nltfflgiis sqasccyail kvknpgmtlk asgsfppeaa hwlcyqafll klaahsviyk
     1081 cllgplrtaq kllcrklpea tmtilkaaad palstdfqti ld
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BLAST COMPARISON — mTRT vs. hTRT

Source: http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi

Score = 1340 bits (3468), Expect = 0.0
Identities = 715/1146 (62%), Positives = 839/1146 (73%), Gaps = 38/1146 (3%)

```
Mouse: 1    MTRAPRCPAVRSLLRSRYREVWPLATFVRRLGPEGRRLVQPGDPKIYRTLVAQCLVCMHW 60
            M RAPRC AVRSLLRS YREV PLATFVRRLGP+G RLVQ GDP  +R LVAQCLVC+ W
Human: 1    MPRAPRCRAVRSLLRSHYREVLPLATFVRRLGPQGWRLVQRGDPAAFRALVAQCLVCVPW 60

Mouse: 61   GSQPPPADLSFHQVSSLKELVARVVQRLCERNERNVLAFGFELLNEARGGPPMAFTSSVR 120
             ++PPPA  SF QVS LKELVARV+QRLCER  +NVLAFGF LL+ ARGGPP AFT+SVR
Human: 61   DARPPPAAPSFRQVSCLKELVARVLQRLCERGAKNVLAFGFALLDGARGGPPEAFTTSVR 120

Mouse: 121  SYLPNTVIETLRVSGAWMLLLSRVGDDLLVYLLAHCALYLLVPPSCAYQVCGSPLYQICA 180
            SYLPNTV + LR SGAW LLL RVGDD+LV+LLA CAL++LV PSCAYQVCG PLYQ+ A
Human: 121  SYLPNTVTDALRGSGAWGLLLRRVGDDVLVHLLARCALFVLVAPSCAYQVCGPPLYQLGA 180

Mouse: 181  TTDIWPSVSASYRPTRPVGRNFTNLRFLQQIKSSSRQEAPKPLALPSRGTKRHLSLTSTS 240
             T   P   AS  P R +G          ++  + S +EA  PL LP+ G +R     S S
Human: 181  ATQARPPPHAS-GPRRRLG--------CERAWNHSVREAGVPLGLPAPGARRRGGSASRS 231

Mouse: 241  VPSAKKARCYPVPRVEEGPHRQVLPTPSGKSWVPSPAR----SPEVPTAEKDLSSKGKVS 296
            +P  K+ R    P  E  P  Q      G++  PS       SP  P AE+  S +G +S
Human: 232  LPLPKRPRRGAAPEPERTPVGQGSWAHPGRTRGPSDRGFCVVSPARP-AEEATSLEGALS 290

Mouse: 297  DLSLSG-SVCCKHKPSSTSLLSPPRQNAFQLRP-FIETRHFLYSRGDGQERLNPSFLLSN 354
                S  SV  +H     S   PPR        P + ET+HFLYS GD +E+L PSFLLS+
Human: 291  GTRHSHPSVGRQHHAGPPSTSRPPRPWDTPCPPVYAETKHFLYSSGD-KEQLRPSFLLSS 349

Mouse: 355  LQPNLTGARRLVEIIFLGSRPRTSGPLCRTHRLSRRYWQMRPLFQQLLVNHAECQYVRLL 414
            L+P+LTGARRLVE IFLGSRP   G   R  RL +RYWQMRPLF +LL NHA+C Y  LL
Human: 350  LRPSLTGARRLVETIFLGSRPWMPGTPRRLPRLPQRYWQMRPLFLELLGNHAQCPYGVLL 409

Mouse: 415  RSHCRFRTA---------------NQQVTDALNTSPPHLMDLLRLHSSPWQVYGFLRACL 459
            ++HC  R A                +    + +T P  L+ LLR HSSPWQVYGF+RACL
Human: 410  KTHCPLRAAVTPAAGVCAREKPQGSVAAPEEEDTDPRRLVQLLRQHSSPWQVYGFVRACL 469

Mouse: 460  CKVVSASLWGTRHNERRFFKNLKKFISLGKYGKLSLQELMWKMKVEDCHWLRSSPGKDRV 519
             ++V   LWG+RHNERRF +N KKFISLGK+ KLSLQEL WKM V DC WLR SPG   V
Human: 470  RRLVPPGLWGSRHNERRFLRNTKKFISLGKHAKLSLQELTWKMSVRDCAWLRRSPGVGCV 529

Mouse: 520  PAAEHRLRERILATFLFWLMDTYVVQLLRSFFYITESTFQKNRLFFYRKSVWSKLQSIGV 579
            PAAEHRLRE ILA FL WLM  YVV+LLRSFFY+TE+TFQKNRLFFYRKSVWSKLQSIG+          22 amino acids
Human: 530  PAAEHRLREEILAKFLHWLMSVYVVELLRSFFYVTETTFQKNRLFFYRKSVWSKLQSIGI 589

Mouse: 580  RQHLERVRLRELSQEEVRHHQDTWLAMPICRLRFIPKPNGLRPIVNMSYSMGTRALGRRK 639
            RQHL+RV+LRELS+ EVR H++   A+   RLRFIPKP+GLRPIVNM Y +G R   R K
Human: 590  RQHLKRVQLRELSEAEVRQHREARPALLTSRLRFIPKPDGLRPIVNMDYVVGARTFRREK 649

Mouse: 640  QAQHFTQRLKTLFSMLNYERTKHPHLMGSSVLGMNDIYRTWRAFVLRVRALDQTPRMYFV 699
            +A+  T R+K LFS+LNYER + P L+G+SVLG++DI+R WR FVLRVRA D  P +YFV
Human: 650  RAERLTSRVKALFSVLNYERARRPGLLGASVLGLDDIHRAWRTFVLRVRAQDPPPELYFV 709

Mouse: 700  KADVTGAYDAIPQGKLVEVVANMIRHSESTYCIRQYAVVRRDSQGQVHKSFRRQVTTLSD 759
            K DVTGAYD IPQ +L EV+A++I+  ++TYC+R+YAVV++ + G V K+F+  V+TL+D
Human: 710  KVDVTGAYDTIPQDRLTEVIASIIK-PQNTYCVRRYAVVQKAAHGHVRKAFKSHVSTLTD 768

Mouse: 760  LQPYMGQFLKHLQDSDASALRNSVVIEQSISMNESSSSLFDFFLHFLRHSVVKIGDRCYT 819
            LQPYM QF+ HLQ++  S LR++VVIEQS S+NE+SS LFD FL F+ H  V+I  + Y
Human: 769  LQPYMRQFVAHLQET--SPLRDAVVIEQSSSLNEASSGLFDVFLRFMCHHAVRIRGKSYV 826

Mouse: 820  QCQGIPQGSSLSTLLCSLCFGDMENKLFAEVQRDGLLLRFVDDFLLVTPHLDQAKTFLST 879
            QCQGIPQGS LSTLLCSLC+GDMENKLFA ++RDGLLLR VDDFLLVTPHL  AKTFL T
Human: 827  QCQGIPQGSILSTLLCSLCYGDMENKLFAGIRRDGLLLRLVDDFLLVTPHLTHAKTFLRT 886
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Mouse: 880  LVHGVPEYGCMINLQKTVVNFPVEPGTLGGAAPYQLPAHCLFPWCGLLLDTQTLEVFCDY 939
            LV GVPEYGC++NL+KTVVNFPVE   LGG A  Q+PAH LFPWCGLLLDT+TLEV  DY
Human: 887  LVRGVPEYGCVVNLRKTVVNFPVEDEALGGTAFVQMPAHGLFPWCGLLLDTRTLEVQSDY 946

Mouse: 940  SGYAQTSIKTSLTFQSVFKAGKTMRNKLLSVLRLKCHGLFLDLQVNSLQTVCINIYKIFL 999
            S YA+TSI+ SLTF   FKAG+ MR KL  VLRLKCH LFLDLQVNSLQTVC NIYKI L
Human: 947  SSYARTSIRASLTFNRGFKAGRNMRRKLFGVLRLKCHSLFLDLQVNSLQTVCTNIYKILL 1006

Mouse: 1000 LQAYRFHACVIQLPFDQRVRKNLTFFLGIISSQASCCYAILKVKNPGMTLKASGS---FP 1056
            LQAYRFHACV+QLPF Q+V KN TFFL +IS  AS CY+ILK KN GM+L A G+    P
Human: 1007 LQAYRFHACVLQLPFHQQVWKNPTFFLRVISDTASLCYSILKAKNAGMSLGAKGAAGPLP 1066

Mouse: 1057 PEAAHWLCYQAFLLKLAAHSVIYKCLLGPLRTAQKLLCRKLPEATMTILKAAADPALSTD 1116
             EA  WLC+QAFLLKL  H V Y  LLG LRTAQ  L RKLP  T+T L+AAA+PAL +D
Human: 1067 SEAVQWLCHQAFLLKLTRHRVTYVPLLGSLRTAQTQLSRKLPGTTLTALEAAANPALPSD 1126

Mouse: 1117 FQTILD 1122
            F+TILD
Human: 1127 FKTILD 1132
```

mine. A single dose of clozapine increases dopamine release in the primate prefrontal cortex, and long-term administration increases basal extracellular dopamine concentration in the prefrontal cortex (*21*). Although this may not be the only mechanism by which clozapine elicits its effects on PCP-induced cognitive dysfunction, this activation of the dopamine system of the prefrontal cortex may contribute to the ability of clozapine to ameliorate the impairments in our model and, perhaps, in schizophrenia.

Our data show that repeated administration of PCP inhibits basal and stimulated dopaminergic function in the prefrontal cortex of the monkey brain. The deficiency of dopamine in the prefrontal cortex that is induced by repeated administration of PCP is associated with a long-lasting cognitive deficit, which is ameliorated by the atypical therapeutic drug clozapine. These effects are observed long after PCP administration is stopped and thus cannot be attributed to direct effects of the drug. This primate model of dopamine dysfunction in the cortex may provide a paradigm for investigating the pathophysiology underlying neuropsychiatric disorders associated with a primary cognitive dysfunction in the cortex and a dopaminergic deficit in the prefrontal cortex, as is hypothesized in schizophrenia (*22*). It also may provide a means for evaluating therapeutic agents that are selectively targeted toward alleviating cortical dopamine hypofunction.

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23. We thank P. S. Goldman-Rakic for critical reading of this manuscript; M. B. Bowers, B. S. Bunney, A. Y. Deutch, G. R. Heninger, and J. F. Tallman for helpful commentary; and A. Tran, Y. Pan, and the staff of the St. Kitts Biomedical Research Foundation for technical support. Supported in part by Public Health Service awards MH14092 and MH44866 (R.H.R.); by the Scottish Rite Schizophrenia Research Program NMJ, USA (J.D.J.); by RSA K05-MH00643 (D.E.R.); and by the Axion Research Foundation.

6 December 1996; accepted 17 June 1997

Telomerase Catalytic Subunit Homologs from Fission Yeast and Human

Toru M. Nakamura, Gregg B. Morin, Karen B. Chapman, Scott L. Weinrich, William H. Andrews, Joachim Lingner,* Calvin B. Harley, Thomas R. Cech†

Catalytic protein subunits of telomerase from the ciliate *Euplotes aediculatus* and the yeast *Saccharomyces cerevisiae* contain reverse transcriptase motifs. Here the homologous genes from the fission yeast *Schizosaccharomyces pombe* and human are identified. Disruption of the *S. pombe* gene resulted in telomere shortening and senescence, and expression of mRNA from the human gene correlated with telomerase activity in cell lines. Sequence comparisons placed the telomerase proteins in the reverse transcriptase family but revealed hallmarks that distinguish them from retroviral and retrotransposon relatives. Thus, the proposed telomerase catalytic subunits are phylogenetically conserved and represent a deep branch in the evolution of reverse transcriptases.

Telomerase is a ribonucleoprotein enzyme responsible in most eukaryotes for the complete replication of chromosome ends, or telomeres (*1*). Its RNA subunit provides the template for addition of short sequence repeats [typically 6 to 26 nucleotides (nts) to the chromosome 3′ end (*2*). In ciliated protozoa and yeast, telomerase is regulated and the average telomere length is maintained (*3*). In most human somatic cells, however, telomerase activity cannot be detected and telomeres shorten with successive cell divisions (*4*). Telomerase activity reappears in immortalized cell lines and in about 85% of human tumors, which has led to studies of the usefulness of telomerase for cancer diagnostics and therapeutics (*5*, *6*).

Telomerase RNA subunits have been identified and analyzed in ciliates, yeast, and mammals (*2*, *7*), but the protein subunits have been elusive. In *Tetrahymena*, two telomerase-associated proteins (p80, p95) have been described (*8*), and p80 homologs have been found in humans and rodents (*9*); the presence of catalytic active site residues in these proteins has not been

established. Purification of telomerase from the ciliate *Euplotes aediculatus* yielded two proteins, p123 and p43 (*10*), that appear unrelated to p80 and p95 (*11*). p123 contains reverse transcriptase (RT) motifs and is homologous to yeast Est2 (Ever shorter telomeres) protein (*11*), which is essential for telomere maintenance in vivo (*12*). The RT motifs of Est2p are essential for telomeric DNA synthesis in vivo and in vitro (*11*, *13*), supporting the conclusion that Est2p and p123 are the catalytic subunits of telomerase. The question remained whether there are two telomerases in biology, one based on p80- and p95-like proteins and one on p123/Est2p-like proteins.

To determine if Est2p/p123 is conserved among eukaryotes, we searched for homologs in the fission yeast *S. pombe* and humans. Polymerase chain reaction (PCR) amplification of *S. pombe* DNA was carried out with degenerate-sequence primers designed from the *Euplotes* p123 RT motifs B′ and C. Of the four prominent products generated, the ~120–base pair (bp) band encoded a peptide sequence homologous to p123 and Est2p. Using this PCR product as a probe for colony hybridization, we identified two overlapping clones from a genomic library and three from a cDNA library (*14*). None of the three cDNA clones was full length, so we used RT-PCR to obtain the NH_2-terminal sequences (*15*). This putative telomerase reverse transcriptase gene, *trt1*$^+$, encoded a basic protein with a predicted molecular mass of 116 kilodaltons (kD) (Fig. 1A). The sequence similarity to p123 and Est2p was especially high in the seven RT motifs (Table 1) and in motif T (Telomerase-specific) (Fig. 2). Fifteen introns, ranging from 36 to 71 bp, interrupted the coding sequence. All had consensus splice and branch site sequences (*16*)..

If *trt1*$^+$ encodes the telomerase catalytic subunit in *S. pombe*, deletion of the gene would be expected to result in telomere shortening and perhaps cellular senescence as seen with the *est2* mutants in *S. cerevisiae* (*11*, *13*). To test this, we created two deletion constructs (Fig. 1A), one removing motifs B′ through E in the RT domain, and the second deleting 99% of the open reading frame (ORF). Haploid cells grown from both types of spores showed progressive telomere shortening to the point where hybridization to telomeric repeats became almost undetectable (Fig. 1B). Senescence was indicated by (i) reduced ability of the cells to grow on agar, typically by the fourth streak-out after germination; (ii) the appearance of colonies with increasingly ragged edges (Fig. 1C); and (iii) the increasing fraction of elongated cells (Fig. 1D). When individual enlarged cells were separated on the dissecting microscope, the majority underwent no further division. The same *trt1*$^-$ cell population always contained normal-size cells that continued to divide but frequently produced nondividing progeny. The telomerase-negative survivors may use a recombinational mode of telomere maintenance as documented in budding yeast strains with deletions of telomere-replication genes (*12*, *17*).

A candidate human p123/Est2p/Trt1p homolog was identified by a BLAST search of the EST (expressed sequence tag) database (GenBank AA281296). This EST was the top-ranked match in sequence searches with *Euplotes* p123 ($P = 3.3 \times 10^{-6}$) and *S. pombe* Trt1p ($P = 9.7 \times 10^{-7}$). The human EST was not found in searches with yeast

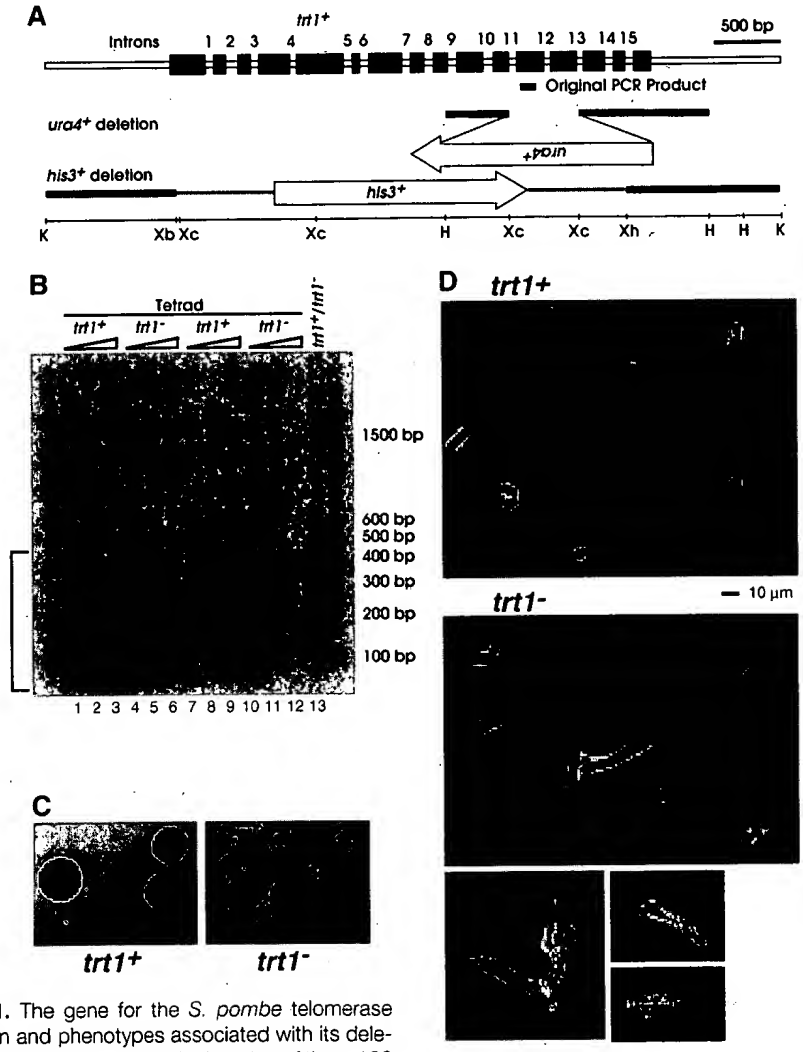

Fig. 1. The gene for the *S. pombe* telomerase protein and phenotypes associated with its deletion. (**A**) The *trt1*$^+$ locus, the location of the ~120 bp PCR product that led to its identification, and the regions replaced by *ura4*$^+$ or *his3*$^+$ genes in the *trt1*$^-$ mutants (K, Kpn I; Xb, Xba I; H, Hind III; Xc, Xca I; Xh, Xho I). (**B**) Telomere shortening phenotype of *trt1*$^-$ mutants. A *trt1*$^+$/*trt1*$^-$ diploid (*28*) was sporulated and the resulting tetrads were dissected and germinated on a YES (Yeast Extract medium Supplemented with amino acids) plate (*29*). Colonies derived from each spore were grown at 32°C for 3 days, and streaked successively to fresh YES plates every 3 days. A colony from each round was placed in 6 ml of YES liquid culture at 32°C and grown to stationary phase, and genomic DNA was prepared. After digestion with Apa I, DNA was subjected to electrophoresis on a 2.3% agarose gel, stained with ethidium bromide to confirm approximately equal loading in each lane, transferred to a nylon membrane, and hybridized to a telomeric DNA probe. The Apa I site is located 30 to 40 bp away from telomeric repeat sequences in chromosomes I and II. (**C**) Colony morphology of *trt1*$^+$ and *trt1*$^-$ cells. Cells plated on MM [Minimal Medium (*29*) with glutamic acid substituted for NH_4Cl] were grown for 2 days at 32°C prior to photography. (**D**) Micrographs of *trt1*$^+$ and *trt1*$^-$ cells grown as in (C).

T. M. Nakamura, J. Lingner, T. R. Cech, Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309–0215, USA.
G. B. Morin, K. B. Chapman, S. L. Weinrich, W. H. Andrews, C. B. Harley, Geron Corporation, 230 Constitution Drive, Menlo Park, CA 94025, USA.
*Present address: Swiss Institute for Experimental Cancer Research, 1066 Epalinges/VD, Switzerland.
†To whom correspondence should be addressed.

Est2p, but subsequent pairwise comparison of these sequences showed a convincing match. Sequencing of the rest of the cDNA clone containing the EST revealed all eight TRT (Telomerase Reverse Transcriptase) motifs, but not in a single ORF (*18*). We used the sequence information from this incomplete cDNA clone to isolate an extended cDNA clone from a library of 293 cells, an adenovirus E1-transformed human embryonic kidney cell line (*19*). This cDNA clone (pGRN121) had a 182-bp insert relative to the EST clone, which increased the spacing between motifs A and B′ (*18*) and put all seven RT motifs and the telomerase-specific motif T motifs in a single contiguous ORF (Fig. 2).

RT-PCR amplification of RNA from 293 cells and from testis each gave two products differing by 182 bp (*20*). The larger and smaller products from testis RNA were sequenced and found to correspond exactly to pGRN121 and the EST cDNA, respectively.

The relative abundance of hTRT mRNA was assessed in six telomerase-negative mortal cell strains and six telomerase-positive immortal cell lines (*21*) (Fig. 3). The steady-state level of hTRT mRNA was higher in immortal cell lines with active telomerase (6) than in any of the telomerase-negative cell strains tested. Telomerase activity was more strongly correlated with the abundance of hTRT mRNA than with that of telomerase RNA (hTR) (*7*). In contrast, the abundance of mRNA for the human p80 homolog TP1 (9) did not correlate with telomerase activity (Fig. 3). Thus, while our proposal that hTRT is the catalytic subunit of human telomerase is based mainly on protein structural features

Table 1. Amino acid sequence identity between telomerase reverse transcriptases. Each value is % identity (% similarity in parentheses) based on RT motifs 1, 2, and A through E in Fig 2C.

	hTRT	SpTrt1p	Est2p
Ea p123	26 (49)	28 (45)	24 (46)
Est2p	25 (46)	27 (48)	
SpTrt1p	30 (47)		

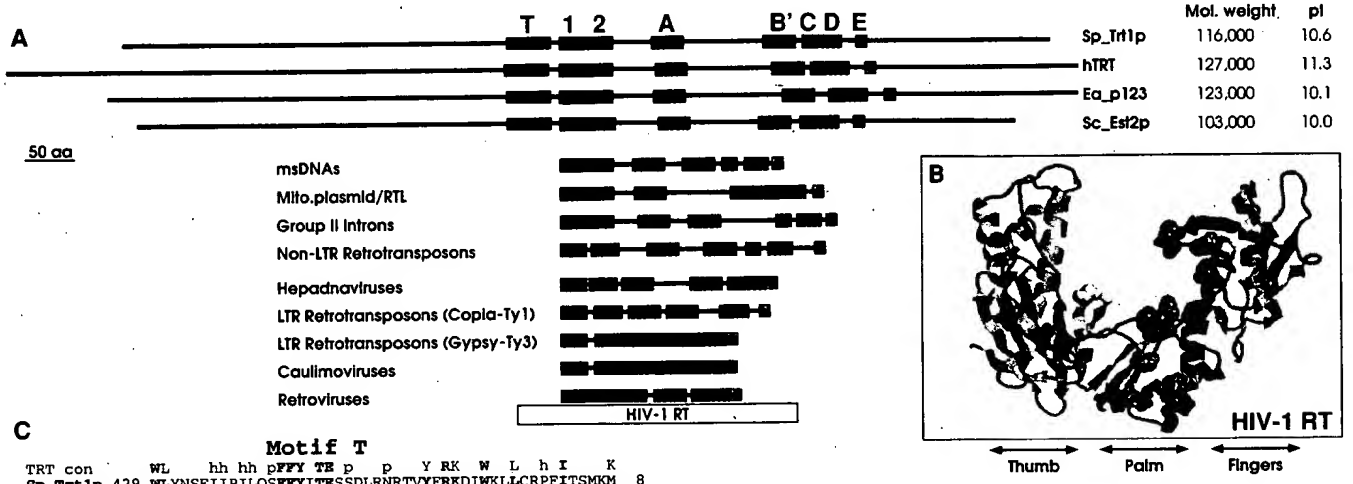

Fig. 2. Structure and RT sequence motifs of telomerase proteins. (**A**) Locations of telomerase-specific motif T and conserved RT motifs 1, 2, and A through E (*24*) are indicated by colored boxes. The open rectangle labeled HIV-1 (Human Immunodeficiency Virus) RT delineates the portion of this protein shown in (B). *pI*, isoelectric point. (**B**) The crystal structure of the p66 subunit of HIV-1 RT (Brookhaven code 1HNV). Color-coding of RT motifs matches that in (A). The view is from the back of the right hand, which allows all motifs to be seen. (**C**) Multiple sequence alignment of telomerase RTs and members of other RT families (Sc_al, cytochrome oxidase group II intron 1-encoded protein from *S. cerevisiae* mitochondria; Dm_TART, reverse transcriptase from *Drosophila melanogaster* TART non-LTR retrotransposable element). Boldface residues indicate identity of at least three telomerase sequences in the alignment. Colored residues are highly conserved in all RTs and shown as space-filled residues in (B). The number of amino acids between adjacent motifs or to the end of the polypeptide is indicated. TRT con and RT con, consensus sequences for telomerase RTs (this work) and non-telomerase RTs (*24*) (amino acids are designated h, hydrophobic, A, L, I, V, P, F, W, M; p, polar, G, S, T, Y, C, N, Q; c, charged, D, E, H, K, R). Red arrowheads show some of the systematic differences between telomerase proteins and other RTs. Red rectangle below motif E highlights the primer grip region discussed in the text. Abbreviations for the amino acids are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. The nucleotide sequences of the *S. pombe trt1*$^+$ gene and the human TRT cDNA (pGRN121) have been deposited in GenBank (accession nos. AF015783 and AF015950, respectively).

(similarity of RT motifs, the T motif, molecular mass > 100 kD, *pI* > 10), the correlation of its mRNA expression level with activity also supports this conclusion.

Sequence alignment of the four telomerase genes revealed features similar to other reverse transcriptases, as well as differences that serve as hallmarks of the telomerase subgroup. The new T motif is one telomerase-specific feature not found in the other RTs examined. Another is the distance between motifs A and B′, which is longer in the TRTs than in other RTs (Fig. 2A). These amino acids can be accommodated as an insertion within the "fingers" region of the structure that resembles a right hand (*22*, *23*) (Fig. 2B). Within the motifs, there are a number of substitutions of amino acids (red arrowheads in Fig. 2C) that are highly conserved among the other RTs. For example, in motif C the two aspartic acid residues (DD) that coordinate active site metal ions (*22*) occur in the context hxDD(F/Y) in the telomerase RTs compared to (F/Y)xDDh in the other RTs (*24*). Another systematic change characteristic of the telomerase subgroup occurs in motif E, where WxGxSx appears to be the consensus among the telomerase proteins, whereas hLGxxh is characteristic of other RTs (*24*). This motif E is called the "primer grip" (*23*), and mutations in this region affect RNA priming but not DNA priming (*25*). Because telomerase uses a DNA primer, the chromosome 3′ end, it is not unexpected that it should differ from other RTs in this region. Given that the simple change from Mg^{2+} to Mn^{2+} allows HIV RT to copy a small region of a template in a repetitive manner (*26*), it is tempting to speculate that some of the distinguishing amino acids in the TRTs may cause telomerase to catalyze repetitive copying of the template sequence of its tightly bound RNA subunit.

Using the seven RT domains (Fig. 2C) defined by Xiong and Eickbush (*24*), we constructed a phylogenetic tree that includes the four telomerase RTs (Fig. 4). The TRTs appear to be more closely related to RTs associated with msDNA (multicopy single-stranded DNA), group II introns, and non-LTR (Long Terminal Repeat) retrotransposons than to the LTR-retrotransposon and viral RTs. The relationship of the telomerase RTs to the non-LTR branch of retroelements is intriguing, given that the latter elements have replaced telomerase for telomere maintenance in *Drosophila* (*27*). However, the most striking finding is that the TRTs form a discrete subgroup, about as closely related to the RNA-dependent RNA polymerases of plus-stranded RNA viruses such as poliovirus as to retroviral RTs. In view of the fact that the four telomerase genes are from evolutionarily distant organisms—protozoan, fungi, and mammals—this separate grouping cannot be explained by lack of phylogenetic diversity in the data set. Instead, this deep branching suggests that the telomerase RTs are an ancient group, perhaps originating with the first eukaryote.

The primary sequence of hTRT and eventual reconstitution of active human telomerase may be used to discover telomerase inhibitors, which in turn will permit additional testing of the anti-tumor effects of telomerase inhibition. The correlation between hTRT mRNA levels and human telomerase activity shown here indicates that hTRT also has promise for cancer diagnosis. With an essential protein component of telomerase now in hand, the stage is set for more detailed investigation of fundamental and applied aspects of this ribonucleoprotein enzyme.

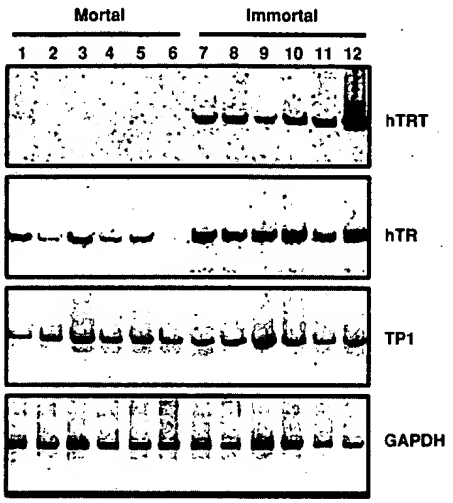

Fig. 3. Expression of hTRT in telomerase-negative mortal cell strains (lanes 1 to 6) and telomerase-positive immortal cell lines (lanes 7 to 12). RT-PCR (*21*) for hTRT, hTR (human telomerase RNA component), TP1 (telomerase-associated protein related to *Tetrahymena* p80), and GAPDH (to normalize for equal amounts of RNA template) was carried out on RNA from: (1) human fetal lung fibroblasts GFL, (2) human fetal skin fibroblasts GFS, (3) adult prostate stromal fibroblasts 31YO, (4) human fetal knee synovial fibroblasts HSF, (5) neonatal foreskin fibroblasts BJ, (6) human fetal lung fibroblasts IMR90, (7) melanoma LOX IMVI (8) leukemia U251, (9) NCI H23 lung carcinoma, (10) colon adenocarcinoma SW620, (11) breast tumor MCF7, and (12) human 293 cells.

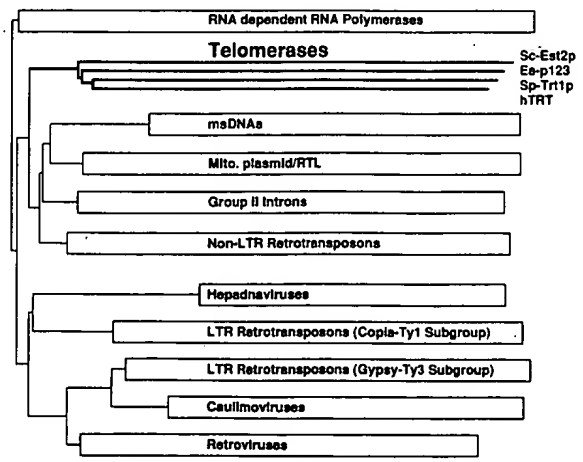

Fig. 4. A possible phylogenetic tree of telomerases and retroelements rooted with RNA-dependent RNA polymerases. After sequence alignment of motifs 1, 2, and A through E (178 positions, Fig. 2C) from four TRTs, 67 RTs, and three RNA polymerases, the tree was constructed using the Neighbor Joining method (*30*). Elements from the same class that are located on the same branch of the tree are simplified as a box. The length of each box corresponds to the most divergent element within that box.

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14. PCR primers were based on motif B′ (YARACHAARGGHATYCCHYARGG) and motif C (NGTNATDARDARRTARTCRTC), where D = G, A, or T; H = T, A, or C; Y = T or C; R = A or G; and N = G, A, T or C. Approximately 10^8 colonies of an *S. pombe* Hind III–cut genomic library in pWH5 [A. Wright, K. Maundrell, W. D. Heyer, D. Beach, P. Nurse, *Plasmid* **15**, 156 (1986)] and 10^5 colonies of an *S. pombe* cDNA library [J.-P. Javerzat, G. Cranston, R. C. Allshire, *Nucleic Acids Res.* **24**, 4676 (1996)] were screened.

15. These cDNA clones terminated in the exon flanked by introns 4 and 5. Poly A+ RNA from *S. pombe* was reverse transcribed using primer M2-B14 (CCTTGGAAAAATCCATTGAAGCCACATGTG). The resulting cDNA was ligated to oligonucleotide pGGGCCGTGTTGGCCTAGTTCTCTGCTCddA using T4 RNA ligase and amplified by two rounds of PCR: in the first round, we used primers M2-B14 and Adapt-Sfi (GAGGAGGAGAAGAGCAGAGAACTAGGCCAACACGGCCC), and in the second, we used primers M2-B15 (AAAGTGGTATGCCAGAAATCTGAAGGTAAT) and Adapt-Sfi.
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19. A lambda cDNA library from the human 293 cell line, which has high levels of telomerase activity, was partitioned into 25 pools containing ~200,000 plaques each. These were screened by PCR with primers LT5 (CGGAAGAGTGTCTGGAGCAA) and LT6 (GGATGAAGCGGAGTCTGGA) to the 5′ region of the #712562 clone insert. Six subpools of one positive primary pool were further screened by PCR. One positive subpool was then screened by plaque hybridization with a probe from the 5′ region of clone #712562. One phage was positively identified and the ~4 kbp insert from this clone was excised and subcloned into the pBluescript II SK+ vector (Stratagene) as an Eco RI fragment.
20. Polyadenylated RNAs from human testis and from the 293 cell line were amplified using a nested PCR strategy. The first primer set was TCP1.1 (GTGAAGGCACTGTTCAGCG) and TCP1.15 (CGCGTGGGTGAGGTGAGGTG); the second primer set was TCP1.14 (CTGTGCTGGGCCTGGACGATA) and bTCP6 (AGCTTGTTCTCCATGTCGCCGTAG).
21. hTRT mRNA was amplified using oligonucleotide primers LT5 and LT6 (*19*) for 31 cycles (94°C for 45 s, 60°C for 45 s, 72°C for 90 s). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was amplified using primers K136 (CTCAGACACCATGGGGAAGGTGA) and K137 (ATGATCTTGAGGCTGTTGTCATA) for 16 cycles (94°C for 45 s, 55°C for 45 s, 72°C for 90 s). hTR was amplified using primers F3b (TCTAACCCTAACTGAGAAGGGCGTAG) and R3c (GTTTGCTCTAGAATGAACGGTGGAAG) for 22 cycles (94°C for 45 s, 55°C for 45 s, 72°C for 90 s). TP1 mRNA was amplified using primers TP1.1 (TCAAGCCAAACCTGAATCTGAG) and TP1.2 (CCCGAGTGAATCTTTCTACGC) for 28 cycles (cycles same as for hTRT). Reaction products were resolved on an 8% polyacrylamide gel, stained with SYBR Green I (Molecular Probes, Eugene, OR).
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23 June 1997; accepted 22 July 1997

Contrasting Genetic Influence of *CCR2* and *CCR5* Variants on HIV-1 Infection and Disease Progression

Michael W. Smith,* Michael Dean,* Mary Carrington,* Cheryl Winkler, Gavin A. Huttley, Deborah A. Lomb, James J. Goedert, Thomas R. O'Brien, Lisa P. Jacobson, Richard Kaslow, Susan Buchbinder, Eric Vittinghoff, David Vlahov, Keith Hoots, Margaret W. Hilgartner, Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study, Stephen J. O'Brien†

The critical role of chemokine receptors (CCR5 and CXCR4) in human immunodeficiency virus–type 1 (HIV-1) infection and pathogenesis prompted a search for polymorphisms in other chemokine receptor genes that mediate HIV-1 disease progression. A mutation (*CCR2-64I*) within the first transmembrane region of the *CCR2* chemokine and HIV-1 receptor gene is described that occurred at an allele frequency of 10 to 15 percent among Caucasians and African Americans. Genetic association analysis of five acquired immunodeficiency syndrome (AIDS) cohorts (3003 patients) revealed that although *CCR2-64I* exerts no influence on the incidence of HIV-1 infection, HIV-1–infected individuals carrying the *CCR2-64I* allele progressed to AIDS 2 to 4 years later than individuals homozygous for the common allele. Because *CCR2-64I* occurs invariably on a *CCR5*-+–bearing chromosomal haplotype, the independent effects of *CCR5-Δ32* (which also delays AIDS onset) and *CCR2-64I* were determined. An estimated 38 to 45 percent of AIDS patients whose disease progresses rapidly (less than 3 years until onset of AIDS symptoms after HIV-1 exposure) can be attributed to their *CCR2*-+/+ or *CCR5*-+/+ genotype, whereas the survival of 28 to 29 percent of long-term survivors, who avoid AIDS for 16 years or more, can be explained by a mutant genotype for *CCR2* or *CCR5*.

The nexus of chemokine immunobiology and AIDS pathogenesis has revealed untapped avenues for resolving patterns of HIV-1 disease progression, for clarifying epidemiologic heterogeneity, and for design of therapies (*1–6*). Identification of the CC-chemokines, RANTES, MIP1α and MIP1β, as suppressor factors produced by CD8 cells that counter infection by certain HIV-1 strain infections (*7*) previewed the critical identification of two chemokine receptor molecules, CXCR4 (formerly named LESTR/fusin) and CCR5 (formerly CKR5), as cell surface coreceptors with CD4 for HIV-1 infection (*8–13*). Additional chemokine receptors CCR2 and CCR3 also have been implicated as HIV-1 coreceptors on certain cell types (*12–14*). HIV-1–infected patients harbor predominantly macrophage-tropic HIV-1 isolates during early stages of infection, but accumulate increasing amounts of T cell–tropic strains just before accelerated T cell depletion and progression to AIDS. The identification of "dual"-tropic HIV-1 strains over the course of infection suggests that such strains may represent an intermediate between macrophage- and T cell–tropic populations (*11–13, 15*). This tropic transition indicates that viral adaptation from CCR5 to CXCR4 receptor use may be a key step in progression to AIDS (*16*).